Document Title

# Monograph of the Ecotoxicological studies of the Plant Production

# Product BYI 02960 480 FS

Data Requirements

**Manual of Requirements and Guidelines, 4th edition/APVMA/Australia**
**Decree 4.074/2002/Brazil**
**Regulatory Directive 2003-01/Canada/PMRA**
**Regulation (EC) No 1107/2009**
**REGULATION in Matter of Registration, Import and Export Authorization and Export Certificates for Pesticides, Nutrients for Plants and Toxic or Dangerous Substances and Materials//SSA-SAGARPA-SEMARNAT/Mexico**
**US EPA OCSPP Guideline Number SUPP**

**Annex III**
**Section 6 Point 10**
**Document M**

**According to OECD format guidance for industry data submissions on plant protection products and their active substances**

Lead Reviewing Agency:

Pest Management Regulatory Agency (Canada)

Secondary Reviewing Agencies:

Australia Pesticide and Veterinary Medicine Authority

United States Environmental Protection Agency

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**Based on original Tier 2 Summary Report prepared by Bayer CropScience (M.G. Dobbs, dated 2012-08-06; Bayer Report No. M-434894-01-1)**

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## KIIIB1 10 Ecotoxicological studies on the plant protection product

The formulation BYI 02960 480 FS (also known as BYI 02960 480 FS G; FS 480) is the seed treatment formulation for the registration and use of the active substance Flupyradifurone (code name: BYI 02960) in Canada and the USA on soybeans.

Only two additional honeybee studies were submitted for this product and are reviewed here.

### KIIIB1 10.4 Effects on bees

### KIIIB1 10.4.1 Acute oral toxicity

| | |
|---|---|
| **Report:** | **KIIIB1 10.4.1/01; Schmitzer, S. (2011)** |
| **Title:** | Effects of BYI 02960 FS 480 G (Acute Contact and Oral) on Honey Bees (*Apis mellifera* L.) in the Laboratory |
| **Report No:** | 64711035 |
| **Document No:** | M-421684-01-3 |
| **Reviewing Agency Document ID** | PMRA #: 2236542<br>APVMA:<br>US EPA: 48844711 |
| **Reviewer Signatures** | PMRA Officer #: 216 (Primary)<br>APVMA: (Secondary)<br>US EPA: (Secondary) |
| **Guidelines:** | OECD Guideline 213<br>OECD Guideline 214 |
| **Deviations:** | For the contact test, a 5 µL droplet was chosen (for any of the treatments) in deviation to the guideline recommendation of 1 µL. The contact test was prolonged for further 48 hours (to 96 hours) due to increased mortality between 24 and 72 hours after test start. |
| **GLP** | Yes (certified laboratory) |
| **PMRA Reliability Rating:** | **Fully Reliable** |
| **APVMA Reliability Rating:** | **NA** (not reviewed because this product will not be used in Australia) |
| **US EPA Reliability Rating:** | **Fully Reliable** |

**EXECUTIVE SUMMARY**

The acute contact and oral toxicity of BYI 02960 FS 480 to the honey bee (*Apis mellifera*) were tested. Thirty honey bees (adult female worker bees) were exposed for 48 hours to dose rates of 5.8, 5.1, 4.0, 2.6 and 1.4 µg a.i./bee via BYI 02960 FS 480 by feeding (oral dose response test, values based on the actual intake of the test item) and 30 worker bees were exposed for 96 hours to dose rates of 200.0, 100.0, 50.0, 25.0 and 12.5 µg a.i./bee via BYI 02960 FS 480 by topical application (contact dose response test).

The contact test was prolonged for further 48 hours due to increased mortality between 24 and 72 hours after test start. In addition, a negative control [tap water (contact test); water/sugar (oral test)] and a toxic reference (Dimethoate; 400 g/L nominal) at nominal rates of 0.30, 0.20, 0.15 and 0.10 µg dimethoate/bee (contact test) and 0.30, 0.15, 0.08 and 0.05 µg dimethoate/bee (oral test), respectively, were tested.

Mortality and abnormal behaviour were recorded 4, 24 and 48 hours after test start (oral and contact test), and in addition 72 and 96 hours after test start (contact test), respectively.

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The toxicity of BYI 02960 FS 480 was tested in both, an acute contact and an acute oral toxicity test on honey bees. The contact $LD_{50}$ values (24, 48, 72 + 96 h) of BYI 02960 FS 480 were determined to be > 200, 137.3, 82.3 and 68.6 µg a.i./bee, respectively. The oral $LD_{50}$ (24 h + 48 h) was 3.4 µg a.i./bee, respectively.

## MATERIAL AND METHODS

### A. Materials

1. Test material

| | |
|---|---|
| Test item: | BYI 02960 FS 480 G |
| Type: | Flowable concentrate for seed treatment |
| Chemical state and description: | Liquid, beige |
| Specification number: | 102000022677-02 |
| Batch No.: | 2010-003945 |
| Material number: | 79911750 |
| Sample description: | TOX09136-00 |
| Nominal content of active ingredient: | BYI 02960: 480 g/L |
| Analytical content of active ingredient: | BYI 02960: 472.6 g/L (39.9% w/w) according to certificate of analysis |
| Solubility: | In water: miscible |
| Density: | 1.186 g/mL (20°C) |
| Stability of test compound: | Expiry date: 03.08.2012, when stored at +2 to +30°C in original container in the dark |

Control

| | |
|---|---|
| Oral Test: | 50% (w/w) aqueous sugar solution (50% tap water, 50% ready-to-use-syrup) |
| Contact Test: | Tap water with 0.5% Adhäsit* (applied after anesthetization with $CO_2$)<br>* (Adhäsit improves spreading of the test droplet on the water-repellent hairs on the thorax of bees) |

Wetting Agent

| | |
|---|---|
| Name: | Adhäsit |
| Batch No.: | 0180201 |
| Analytical content of active ingredient: | 100 g/L Marlopon (nominal) |
| Type: | Adhesive |
| Manufacturer: | Spiess-Urania Chemicals GmbH,<br>Heidenkampsweg 77, 20097 Hamburg, Germany |
| Storage: | Expiry Date: 02/2013, when stored in original container, at room temperature (20 ± 5°C), in the dark |
| Target Amount in this Study: | 0.5% |

Reference Item

The information concerning the reference item according to the substance container label and data sheet:

| | |
|---|---|
| Name: | Perfekthion EC (BAS 152 11 I) |
| Manufacturer: | BASF AG, Agricultural Center Limburgerhof, D-67114 Limburgerhof |
| Batch No.: | 90924-06 |
| Nominal content of active ingredient: | Dimethoate: 400 g/L |
| Analytical content of active ingredient: | Dimethoate: 414.8 g/L according to certificate of analysis |
| Certificate of Analysis Study Code: | 346282_32 |
| Type of formulation: | EC |
| Chemical state and description: | Liquid, blue |
| Density: | 1.074 g/cm$^3$ |
| Solubility: | In water: emulsifiable |
| Stability: | Expiry date: 07.10.2011, when stored in original container, at ≤10°C, in the dark |

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2. Test organisms

| | |
|---|---|
| Species: | *Apis mellifera carnica* L. |
| Common name: | Honey bee |
| Age or developmental stage at test start: | Adult female worker bees |
| Source: | Honey bee colonies, disease-free and queen-right, bred by IBACON |

**B. Study design and methods**

1. Experimental treatments:

Test units were stainless steel chambers of 10 cm x 8.5 cm x 5.5 cm (length x width x height), the front side was a removable glass sheet, the bottom was perforated with 98 ventilation holes (Ø 1 mm), the inner walls were lined with filter paper.

10 bees were used per replicate unit, 3 replicates per treatment group (i.e. 30 individuals per treatment group).
Exposure time was 96 hours for the contact test (which was prolonged due to increasing mortality between 24 and 72 hours) and 48 hours for the oral test, respectively.

As food, commercial ready-to-use syrup (Apiinvert; 30% sucrose, 31% glucose, 39% fructose) was offered to the bees *ad libitum*. The syrup was administered using syringes directly after the treatment solutions had been consumed. This was done with syringes that were inserted into the cages *via* an opening in the top of the test units and from which bees accessed the food directly. Fresh syrup was supplied after 48 hours (contact test).

Bees in the oral test were starved for 20 minutes prior to test start at all treatment groups.

Bees in the contact test were anesthetized for ca. 20 seconds with $CO_2$ until they were completely immobilized immediately before application (only in the contact test).

Test units were uniquely identified with study number, application date, treatment, concentration and replicate number.

Control:
Contact test: $CO_2$/tap water + Adhäsit[1]
Oral test: Aqueous sugar solution

Test item:
Contact Test:

| | |
|---|---|
| Nominal dose levels | 200.0, 100.0, 50.0, 25.0 and 12.5 µg a.i./bee via BYI 02960 FS 480 |

Oral Test:

| | |
|---|---|
| Nominal dose levels | 20.0, 10.0, 5.0, 2.5 and 1.3 µg a.i./bee via BYI 02960 FS 480 |
| Measured dose levels | 5.8, 5.1, 4.0, 2.6 and 1.4 µg a.i./bee via BYI 02960 FS 480 |

[1] Adhäsit was used to improve the spreading of the test droplet on the bee body. Adhäsit is non-toxic to honey bees.

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Toxic reference item:
Contact test:
Nominal dose levels 0.30, 0.20, 0.15 and 0.10 µg Dimethoate per bee
Oral Test:
Nominal dose levels 0.30, 0.15, 0.08 and 0.05 µg Dimethoate per bee
Measured dose levels 0.33, 0.16, 0.08 and 0.05 µg Dimethoate per bee

Application of the test item in the contact test:

Bees were anaesthetized with $CO_2$ in the contact test. A single 5 µL droplet of BYI 02960 FS 480 in an appropriate carrier (tap water and 0.5% Adhäsit) was placed on the dorsal bee thorax using a Burkard - Applicator. For the control, one 5 µL droplet of tap water with 0.5% Adhäsit was used. The reference item was also applied in a 5 µL droplet (dimethoate made up in tap water containing 0.5% Adhäsit).

A 5 µL droplet was chosen in deviation to the guideline recommendation of 1 µL, since a higher volume ensured a more reliable dispersion of the test item; IBACON experience has proven that higher volumes are suitable and no adverse effects on the outcome of the study are to be expected.

Application of the test item in the oral test:

Aqueous stock solutions of the test item and reference item were prepared in order to achieve the target concentrations after being mixed with sugar syrup (ready-to-use syrup; Apiinvert, Südzucker, D-97195 Ochsenfurt at a ratio of 1 : 1. After mixing of these test or reference item solutions with ready-to-use sugar syrup, the final concentration of sugar syrup in the test and reference item solutions offered to the bees was 50% (50% aqueous test or reference item solution and 50% syrup (w/w)). For the control, water and sugar syrup was used at the same ratio (50% water and 50% syrup (w/w)).

The treated food was offered in syringes, which were weighed before and after introduction into the cages (duration of uptake ranged from 0.5 to 6 hours for the test item treatments). After a maximum of 6 hours, the syringes containing the treated food were removed, weighed and replaced by ones containing fresh, untreated food.

The mean target dose levels (*e.g.* 20 µg a.i./bee nominal) would have been obtained if exactly 20 mg/bee of the treated food were ingested. In practice, uptake of the treated sugar solutions differed from nominal and as such, results are given based on the measured consumption.

2. Observation and measurements:

The number of dead bees was determined after 4 (± 0.5) hours (first day); 24 and 48 (± 2) hours (contact and oral test); and additionally after 72 and 96 (± 2) hours (contact test).

Behavioural abnormalities (vomiting, apathy, intensive cleaning) were assessed after 4 (± 0.5) hours (first day); 24 and 48 hours (contact and oral test); and additionally after 72 and 96 (± 2) hours (contact test).

Result evaluation:

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Results obtained with the bees treated with the test item and the reference item were compared to those obtained with the control in both the contact and oral tests.
The contact and oral $LD_{50+20+10}$ values of the test item were estimated with Probit Analysis (according to Finney 1971).
The contact and oral $LD_{50}$ values of the reference item were estimated using the binomial distribution (according to Stephan, 1977).

It was not necessary to correct the $LD_{50}$ calculation by control mortality using Abbott's formula (1925), because there was no control mortality.

The NOED was estimated using Fisher Exact Test (pairwise comparison, one-sided greater, $\alpha = 0.05$), which is a distribution-free test and does not require testing for normality or homogeneity prior to analysis.

The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

## RESULTS AND DISCUSSION

### A. Environmental Parameters

Measurements of climatic parameters during the test are summarized as follows:

| | |
|---|---|
| Test environment: | Incubator |
| Test temperature: | 25°C; short-term deviations (< 2 hours) are not reported |
| Relative air humidity: | 51 to 84%; short-term deviations (< 2 hours) are not reported |
| Light intensity: | Darkness (except during observation) |
| Ventilation: | Ventilation to avoid possible accumulation of pesticide vapour |
| Recording: | Test conditions were continuously recorded with electronic data logger and documented in the raw data |

### B. Biological Findings

**Contact Test:**

The contact test was prolonged for a further 48 hours up to 96 hours due to increasing mortality between 24/48 and 48/72 hours. Dose levels of 200.0, 100.0, 50.0, 25.0 and 12.5 µg a.i./bee resulted in mortality of 70.0, 73.3, 53.3, 10.0 and 6.7% at test termination (96 hours after application). No mortality occurred in the control group (water + 0.5% Adhäsit).

During the first 48 hours of the test, a few bees were behaving abnormal amongst the treatment groups (*e.g.* movement coordination problems and/or apathy). No behavioural abnormalities occurred anymore during the 72 and 96-hrs assessments, respectively. In the 25.0 µg/bee dose level, all bees behaved normal during the entire time of the experiment.

**Oral Test:**

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In the oral test, the three highest nominal dose levels of the test item corresponding to 20.0, 10.0 and 5.0 µg a.i./bee could not be achieved, because the bees did not ingest the full volume of treated sugar solution even when offered over a period of 6 hours. Actual oral doses of 5.8, 5.1, 4.0 and 2.6 µg a.i./bee resulted in mortality ranging from 93.3% to 23.3% at the end of the test (48 hours after application).
No mortality occurred in the 1.4 µg a.i./bee group and in the control group, respectively.

During the 4 hours assessment movement coordination problems and/or apathy were observed in the four highest dose groups (5.8, 5.1, 4.0 and 2.6 µg a.i./bee). After 24 hours, dis-coordinated movements and intensive cleaning of a few bees were found in the 5.8 and 5.1 µg a.i./bee groups. 48 hours following the application the bees behaved normal. No further behavioural abnormalities occurred.

## C. Validity Criteria

The validity criterion of control mortality <10% is fulfilled; the validity criterion regarding the performance of the toxic reference is fulfilled for both contact and oral toxicity test, respectively.

## D. Biological Endpoints Derived

From the results presented above the following biological endpoints can be derived:

**Table 1: Toxicity to Honey Bees; laboratory tests**

| **Test Item** | **BYI 02960 FS 480** | |
|---|---|---|
| **Test Object** | ***Apis mellifera*** | |
| **Exposure** | **contact (solution in Adhäsit (0.5 %)/water)** | **oral (sugar solution)** |
| **Application rate µg a.i./bee** | **200.0, 100.0, 50.0, 25.0 and 12.5** | **5.8, 5.1, 4.0, 2.6 and 1.4** |
| $LD_{50}$ µg a.i./bee | 24 hours: > 200.0;<br>48 hours: 137.3;<br>72 hours: 82.3;<br>96 hours: 68.6 | 24 hours: 3.4;<br>48 hours: 3.4 |
| $LD_{20}$ µg a.i./bee | 24 hours: 64.0;<br>48 hours: 35.8;<br>72 hours: 27.6;<br>96 hours: 25.0 | 24 hours: 2.5;<br>48 hours: 2.5 |
| $LD_{10}$ µg a.i./bee | 24 hours: 25.1;<br>48 hours: 17.7;<br>72 hours: 15.6;<br>72 hours: 14.7 | 24 hours: 2.1;<br>48 hours: 2.1 |
| NOED µg a.i./bee* | 24 hours: 25.0;<br>48 hours: 25.0;<br>72 hours: 25.0;<br>72 hours: 25.0 | 24 hours: 1.4;<br>48 hours: 1.4 |

* The NOED was estimated using Fisher Exact Test (pairwise comparison, one-sided greater, $\alpha = 0.05$).

**<u>Contact toxicity test:</u>**

The contact $LD_{50}$ values (24, 48, 72 + 96 h) of BYI 02960 FS 480 were determined to be > 200, 137.3, 82.3 and 68.6 µg a.i./bee, respectively.

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**Table 2: Mortality and behavioural abnormalities of the bees in the contact toxicity test**

| | after 24 hours | | after 48 hours | | after 72 hours | | after 96 hours | |
|---|---|---|---|---|---|---|---|---|
| | mortality | behav. abnorm. | mortality | behav. abnorm. | mortality | behav. abnorm. | mortality | behav. abnorm. |
| dosage | mean | mean | mean | mean | mean | mean | mean | mean |
| [µg a.i./bee] | % | % | % | % | % | % | % | % |
| test item | | | | | | | | |
| 200.0 | 40.0 | 3.3 | 50.0 | 10.0 | 66.7 | 0.0 | 70.0 | 0.0 |
| 100.0 | 13.3 | 3.3 | 43.3 | 3.3 | 60.0 | 0.0 | 73.3 | 0.0 |
| 50.0 | 33.3 | 3.3 | 46.7 | 3.3 | 53.3 | 0.0 | 53.3 | 0.0 |
| 25.0 | 6.7 | 0.0 | 6.7 | 0.0 | 6.7 | 0.0 | 10.0 | 0.0 |
| 12.5 | 3.3 | 0.0 | 3.3 | 3.3 | 6.7 | 0.0 | 6.7 | 0.0 |
| water | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| reference item | | | | | | | | |
| 0.30 | 76.7 | 3.3 | 83.3 | 0.0 | 83.3 | 0.0 | 83.3 | 0.0 |
| 0.20 | 80.0 | 0.0 | 86.7 | 3.3 | 86.7 | 0.0 | 86.7 | 0.0 |
| 0.15 | 40.0 | 3.3 | 50.0 | 0.0 | 53.3 | 0.0 | 63.3 | 0.0 |
| 0.10 | 3.3 | 10.0 | 13.3 | 10.0 | 20.0 | 0.0 | 23.3 | 0.0 |

results are averages from three replicates (ten bees each) per dosage/control
see Appendix 1, Table 4 for details
behav. abnorm. = behavioural abnormalities; water = $CO_2$/water-treated control

## Oral toxicity test:

The oral $LD_{50}$ (24 + 48 h) was determined to be 3.4 µg a.i./bee, respectively.

**Table 3: Mortality and behavioural abnormalities of the bees in the oral toxicity test**

| | after 4 hours | | after 24 hours | | after 48 hours | |
|---|---|---|---|---|---|---|
| | mortality | behav. abnorm. | mortality | behav. abnorm. | mortality | behav. abnorm. |
| uptaken dosage | mean | mean | mean | mean | mean | mean |
| [µg a.i./bee] | % | % | % | % | % | % |
| test item | | | | | | |
| 5.8 | 56.7 | 43.3 | 93.3 | 3.3 | 93.3 | 0.0 |
| 5.1 | 43.3 | 56.7 | 76.7 | 6.7 | 76.7 | 0.0 |
| 4.0 | 36.7 | 53.3 | 76.7 | 0.0 | 76.7 | 0.0 |
| 2.6 | 13.3 | 23.3 | 23.3 | 0.0 | 23.3 | 0.0 |
| 1.4 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| water | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| reference item | | | | | | |
| 0.33 | 23.3 | 33.3 | 100.0 | 0.0 | 100.0 | 0.0 |
| 0.16 | 0.0 | 6.7 | 36.7 | 13.3 | 70.0 | 0.0 |
| 0.08 | 0.0 | 0.0 | 3.3 | 3.3 | 13.3 | 3.3 |
| 0.05 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

results are averages from three replicates (ten bees each) per dosage/control
see Appendix 1, Table 6 for details
behav. abnorm. = behavioural abnormalities

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The $LD_{50}$ (24 h) values of the reference item (dimethoate) were calculated to be 0.16 (contact test) and 0.19 µg a.i./bee (oral test), respectively.

**Study Reliability and Discussion of Deficiencies**

This study is considered fully reliable. No deficiency was noted.

Endpoint calculations were verified (Appendix 1).

**CONCLUSION**

The toxicity of BYI 02960 FS 480 was tested in both, an acute contact and an acute oral toxicity test on honey bees. The contact $LD_{50}$ values (24, 48, 72 + 96 h) of BYI 02960 FS 480 were determined to be > 200, 137.3, 82.3 and 68.6 µg a.i./bee, respectively. The oral $LD_{50}$ (24 h + 48 h) was 3.4 µg a.i./bee, respectively.

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Appendix 1:

# Statistical Evaluation of a Quantal Response: M-421684-01-3 (PMRA # 2236542)

**General:**

| | |
|---|---|
| Test identification/project no. | M-421684-01-3 (PMRA # 2236542) |
| Test item | BYI 02960 SL 200 G |
| Unit of test item concentration | µg a.i./bee |
| Start of experiment on day | |
| Date and time of the evaluation | 2013-05-03; 2:37:51 PM |
| Raw data filename: | 48 Hour Contact Bee toxicity 2236542_2004-3146.xls |

**Test design**

| | |
|---|---|
| Number of treatments (incl. control(s)) | 6 |
| Duration of the test | 48 h |
| Test system | |

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### Overview Mortality

Tab. 1: % Mortality caused by the test item at 48 h.

| Treatm.[µg a.i./bee] | %Introduced | %Survived | %Dead |
|---|---|---|---|
| Control | 100.0 | 100.0 | 0.0 |
| 12.000 | 100.0 | 96.7 | 3.3 |
| 25.000 | 100.0 | 93.3 | 6.7 |
| 50.000 | 100.0 | 53.3 | 46.7 |
| 100.000 | 100.0 | 56.7 | 43.3 |
| 200.000 | 100.0 | 50.0 | 50.0 |

## Effective Concentrations (ECx) for Mortality at 48 h

### Probit analysis using simple linear regression

Tab. 2: Probit analysis using simple linear regression: Determination of the concentration/response function; data is shown which entered the probit analysis; Log(x): logarithm of the concentration; n: number of replicates; Emp. Probit: empirical probit; Reg. Probit: calculated probit for the final function.

| Treatm. [µg a.i./bee] | Log(x) | % Mortality | n | Emp. Probit |
|---|---|---|---|---|
| Control | | 0.00 | 1 | |
| 12.000 | 1.079 | 3.30 | 1 | -1.8386 |
| 25.000 | 1.398 | 6.70 | 1 | -1.4977 |
| 50.000 | 1.699 | 46.70 | 1 | -0.0819 |
| 100.000 | 2.000 | 43.30 | 1 | -0.1672 |
| 200.000 | 2.301 | 50.00 | 1 | 0.0000 |

excluded: value not in line with the chosen function

### Parameters of the probit analysis

Tab. 3: Parameters of the probit analysis: Results of the regression analysis

| Parameter | Value |
|---|---|
| Computation runs: | 0 |
| Slope b: | 1.64624 |
| Intercept a: | -3.50816 |
| Variance of b: | 1.07783 |
| Goodness of Fit | |
| Chi²: | 0.57279 |
| Degrees of freedom: | 3 |
| p(Chi²): | 0.90263 |
| Log EC50: | 2.13102 |
| SE Log EC50: | 0.38634 |
| g-Criterion: | 1.52784 |
| F: | 13.169 |
| p(F) (df: 1;3): | 0.036 |

Chi² is a goodness of fit measure. If the probability, p(Chi²), is lower or equal than 0.100, data is much scattering round the computed dose/response function. In this case and with quantal data, confidence limits are corrected for heterogeneity (= are made wider; so, check whether these results are reasonable!).

### Results of the probit analysis

Tab. 4: Results of the probit analysis: Selected effective concentrations (ECx) of the test item and their 95%- and 99%-confidence limits (according to Fieller`s theorem).

| Parameter | EC50 |
|---|---|
| Value [µg a.i./bee] | 135.213 |
| lower 95%-cl | n.d. |

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| | |
|---|---|
| upper 95%-cl | n.d. |
| lower 99%-cl | n.d. |
| upper 99%-cl | n.d. |

n.d.: not determined due to mathematical reasons or inappropriate data

Slope function after Litchfield and Wilcoxon: 4.050

(The slope function is derived from the slope, b, of the linearized probit function and computes as S = 10^(1/b); please note that small values refer to a steep concentration/response relation and large ones to a flat relation.)

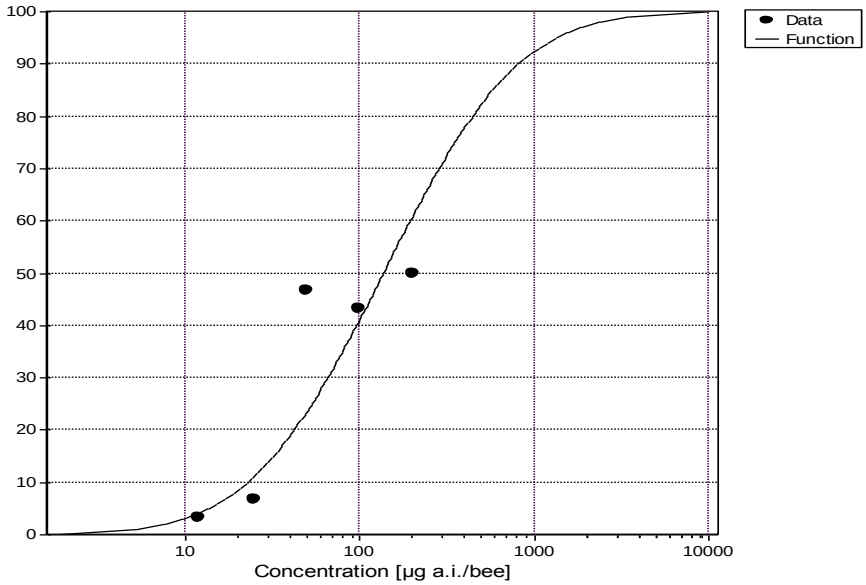


Fig. 1: Concentration-effect curve showing the influence of the test item on mortality of the introduced test organism as observed after 48 h.

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# Statistical Evaluation of a Quantal Response: M-421684-01-3 (PMRA # 2236542)

**General:**

| | |
|---|---|
| Test identification/project no. | M-421684-01-3 (PMRA # 2236542) |
| Test item | BYI 02960 SL 200 G |
| Unit of test item concentration | µg a.i./bee |
| Start of experiment on day | |
| Date and time of the evaluation | 2013-05-03; 2:42:10 PM |
| Raw data filename: | 96 Hour Contact Bee toxicity 2236542_2004-3146.xls |

**Test design**

| | |
|---|---|
| Number of treatments (incl. control(s)) | 6 |
| Duration of the test | 96 h |
| Test system | |

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### Overview Mortality

Tab. 1: % Mortality caused by the test item at 96 h.

| Treatm.[µg a.i./bee] | %Introduced | %Survived | %Dead |
|---|---|---|---|
| Control | 100.0 | 100.0 | 0.0 |
| 12.000 | 100.0 | 93.3 | 6.7 |
| 25.000 | 100.0 | 90.0 | 10.0 |
| 50.000 | 100.0 | 46.7 | 53.3 |
| 100.000 | 100.0 | 26.7 | 73.3 |
| 200.000 | 100.0 | 30.0 | 70.0 |

## Effective Concentrations (ECx) for Mortality at 96 h

### Probit analysis using simple linear regression

Tab. 2: Probit analysis using simple linear regression: Determination of the concentration/response function; data is shown which entered the probit analysis; Log(x): logarithm of the concentration; n: number of replicates; Emp. Probit: empirical probit; Reg. Probit: calculated probit for the final function.

| Treatm. [µg a.i./bee] | Log(x) | % Mortality | n | Emp. Probit |
|---|---|---|---|---|
| Control | | 0.00 | 1 | |
| 12.000 | 1.079 | 6.70 | 1 | -1.4977 |
| 25.000 | 1.398 | 10.00 | 1 | -1.2801 |
| 50.000 | 1.699 | 53.30 | 1 | 0.0819 |
| 100.000 | 2.000 | 73.30 | 1 | 0.6191 |
| 200.000 | 2.301 | 70.00 | 1 | 0.5216 |

excluded: value not in line with the chosen function

### Parameters of the probit analysis

Tab. 3: Parameters of the probit analysis: Results of the regression analysis

| Parameter | Value |
|---|---|
| Computation runs: | 0 |
| Slope b: | 1.94931 |
| Intercept a: | -3.61593 |
| Variance of b: | 1.07783 |
| Goodness of Fit | |
| Chi²: | 0.53493 |
| Degrees of freedom: | 3 |
| p(Chi²): | 0.91115 |
| Log EC50: | 1.85498 |
| SE Log EC50: | 0.24465 |
| g-Criterion: | 1.08969 |
| F: | 19.771 |
| p(F) (df: 1;3): | 0.021 |

Chi² is a goodness of fit measure. If the probability, p(Chi²), is lower or equal than 0.100, data is much scattering round the computed dose/response function. In this case and with quantal data, confidence limits are corrected for heterogeneity (= are made wider; so, check whether these results are reasonable!).

### Results of the probit analysis

Tab. 4: Results of the probit analysis: Selected effective concentrations (ECx) of the test item and their 95%- and 99%-confidence limits (according to Fieller`s theorem).

| Parameter | EC50 |
|---|---|
| Value [µg a.i./bee] | 71.612 |
| lower 95%-cl | n.d. |

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| | |
|---|---|
| upper 95%-cl | n.d. |
| lower 99%-cl | n.d. |
| upper 99%-cl | n.d. |

n.d.: not determined due to mathematical reasons or inappropriate data

Slope function after Litchfield and Wilcoxon: 3.258

(The slope function is derived from the slope, b, of the linearized probit function and computes as S = 10^(1/b); please note that small values refer to a steep concentration/response relation and large ones to a flat relation.)

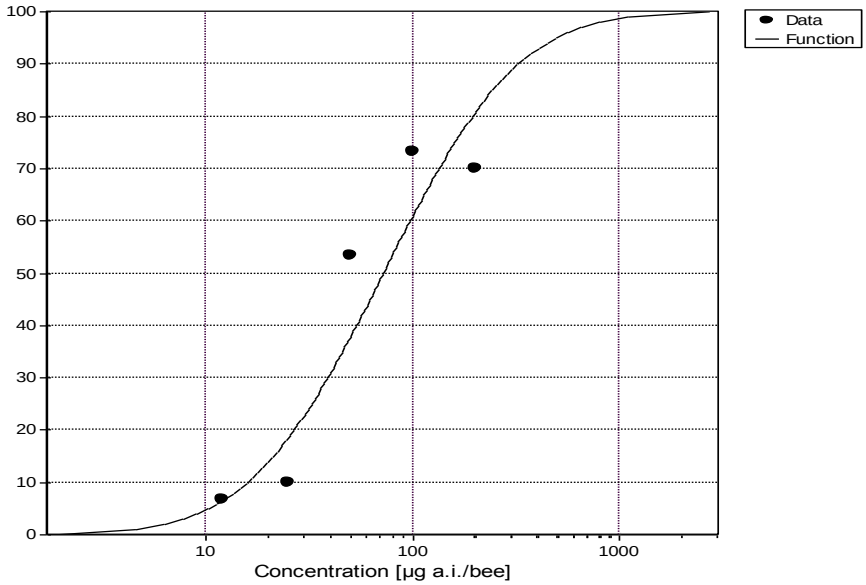


Fig. 1: Concentration-effect curve showing the influence of the test item on mortality of the introduced test organism as observed after 96 h.

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# Statistical Evaluation of a Quantal Response: M-421684-01-3 (PMRA # 2236542)

**General:**

| | |
|---|---|
| Test identification/project no. | M-421684-01-3 (PMRA # 2236542) |
| Test item | BYI 02960 480 FS |
| Unit of test item concentration | µg a.i./bee |
| Start of experiment on day | |
| Date and time of the evaluation | 2013-05-03; 2:08:00 PM |
| Raw data filename: | 48 Hour Oral Bee toxicity 2236542_2004-3146.xls |

**Test design**

| | |
|---|---|
| Number of treatments (incl. control(s)) | 6 |
| Duration of the test | 48 h |
| Test system | |

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### Overview Mortality

Tab. 1: % Mortality caused by the test item at 48 h.

| Treatm.[µg a.i./bee] | %Introduced | %Survived | %Dead |
|---|---|---|---|
| Control | 100.0 | 100.0 | 0.0 |
| 1.400 | 100.0 | 100.0 | 0.0 |
| 2.600 | 100.0 | 76.7 | 23.3 |
| 4.000 | 100.0 | 23.3 | 76.7 |
| 5.100 | 100.0 | 23.3 | 76.7 |
| 5.800 | 100.0 | 6.7 | 93.3 |

## Effective Concentrations (ECx) for Mortality at 48 h

### Probit analysis using simple linear regression

Tab. 2: Probit analysis using simple linear regression: Determination of the concentration/response function; data is shown which entered the probit analysis; Log(x): logarithm of the concentration; n: number of replicates; Emp. Probit: empirical probit; Reg. Probit: calculated probit for the final function.

| Treatm. [µg a.i./bee] | Log(x) | % Mortality | n | Emp. Probit |
|---|---|---|---|---|
| Control | | 0.00 | 1 | |
| 1.400 | 0.146 | 0.00 | 1 | -3.7218 |
| 2.600 | 0.415 | 23.30 | 1 | -0.7263 |
| 4.000 | 0.602 | 76.70 | 1 | 0.7263 |
| 5.100 | 0.708 | 76.70 | 1 | 0.7263 |
| 5.800 | 0.763 | 93.30 | 1 | 1.4977 |

excluded: value not in line with the chosen function

### Parameters of the probit analysis

Tab. 3: Parameters of the probit analysis: Results of the regression analysis

| Parameter | Value |
|---|---|
| Computation runs: | 0 |
| Slope b: | 8.09689 |
| Intercept a: | -4.56525 |
| Variance of b: | 3.97218 |
| Goodness of Fit | |
| Chi²: | 0.72396 |
| Degrees of freedom: | 3 |
| p(Chi²): | 0.86755 |
| Log EC50: | 0.56383 |
| SE Log EC50: | 0.05598 |
| g-Criterion: | 0.23276 |
| F: | 68.393 |
| p(F) (df: 1;3): | 0.004 |

Chi² is a goodness of fit measure. If the probability, p(Chi²), is lower or equal than 0.100, data is much scattering round the computed dose/response function. In this case and with quantal data, confidence limits are corrected for heterogeneity (= are made wider; so, check whether these results are reasonable!).

### Results of the probit analysis

Tab. 4: Results of the probit analysis: Selected effective concentrations (ECx) of the test item and their 95%- and 99%-confidence limits (according to Fieller`s theorem).

| Parameter | EC50 |
|---|---|
| Value [µg a.i./bee] | 3.663 |
| lower 95%-cl | 2.814 |

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| | |
|---|---|
| upper 95%-cl | 5.021 |
| lower 99%-cl | 2.569 |
| upper 99%-cl | 5.500 |

n.d.: not determined due to mathematical reasons or inappropriate data

Slope function after Litchfield and Wilcoxon: 1.329

(The slope function is derived from the slope, b, of the linearized probit function and computes as S = 10^(1/b); please note that small values refer to a steep concentration/response relation and large ones to a flat relation.)

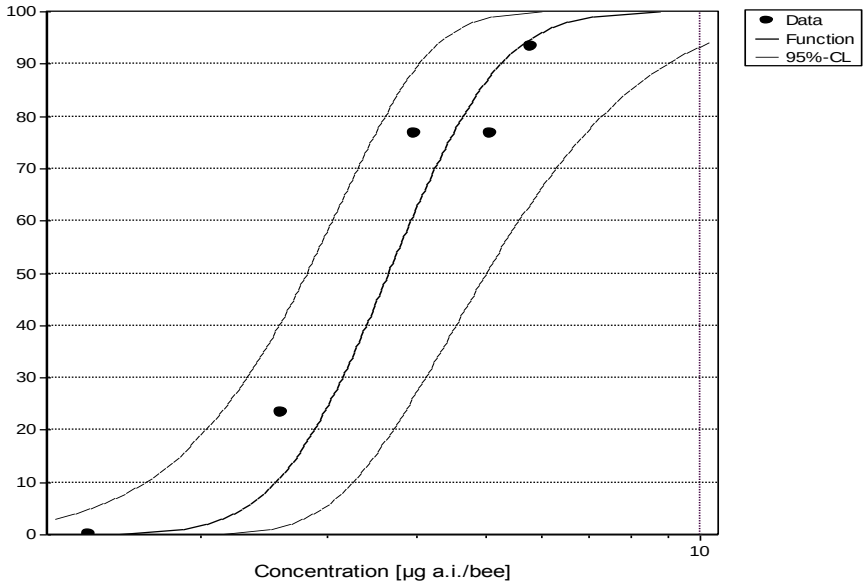


Fig. 1: Concentration-effect curve showing the influence of the test item on mortality of the introduced test organism as observed after 48 h.

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## KIIIB1 10.4.5 Field Tests

The following two studies were reviewed under KIIIA1 10.4.5/03 and KIIIA1 10.4.5/04.

| | |
|---|---|
| **Report:** | **KIIIB1 10.4.5/01; KIIIA1 10.4.5/03; Rexer, H.-U., 2012** |
| **Title:** | A Field Study to Determine Residues of BYI 02960 in Guttation Liquid from Winter Oil-Seed Rape (OSR) Plants in Northern Germany in 2010/2011 |
| **Study No:** | S10-03312 |
| **Document No:** | M-438826-01-2 |
| **Reviewing Agency**<br>**Document ID** | PMRA: 2236676<br>APVMA: 70825<br>US EPA: 48844537 |
| **Reviewer Signatures** | PMRA Officer #: 1184 (Primary)<br>APVMA: (Secondary)<br>US EPA: (Secondary) |
| **Guidelines:** | IVA (Beutel et al., 1992); Commission Directive 96/68/EC (1996), Working document 1607/VI/97 rev.1 (1997); OCSPP 850.SUPP |
| **Deviations:** | None |
| **GLP:** | Yes (except weather data from official weather station and farmer information) |
| **PMRA Reliability Rating:** | **Fully Reliable** |
| **APVMA Reliability Rating:** | **Fully Reliable** |
| **US EPA Reliability Rating:** | **Reliable with Restrictions** |

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|---|---|
| **Report:** | **KIIIB1 10.4.5/02; KIIIA1 10.4.5/04; Rexer, H.-U., 2012** |
| **Title:** | A Field Study to Determine Residues of BYI 02960 in Guttation Liquid from Winter Oil-Seed Rape (OSR) Plants in France in 2010/2011 |
| **Study No:** | S10-03313 |
| **Document No:** | M-438829-01-2 |
| **Reviewing Agency**<br>**Document ID** | PMRA: 2236677<br>APVMA: 70826<br>US EPA: 48844538 |
| **Reviewer Signatures** | PMRA Officer #: 1184 (Primary)<br>APVMA: (Secondary)<br>US EPA: (Secondary) |
| **Guidelines:** | **IVA (Beutel et al., 1992); Commission Directive 96/68/EC (1996), Working document 1607/VI/97 rev.1 (1997); OCSPP 850.SUPP** |
| **Deviations:** | **None** |
| **GLP:** | **Yes** |
| **PMRA Reliability Rating:** | **Fully Reliable** |
| **APVMA Reliability Rating:** | **Fully Reliable** |
| **US EPA Reliability Rating:** | **Reliable with Restrictions** |

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